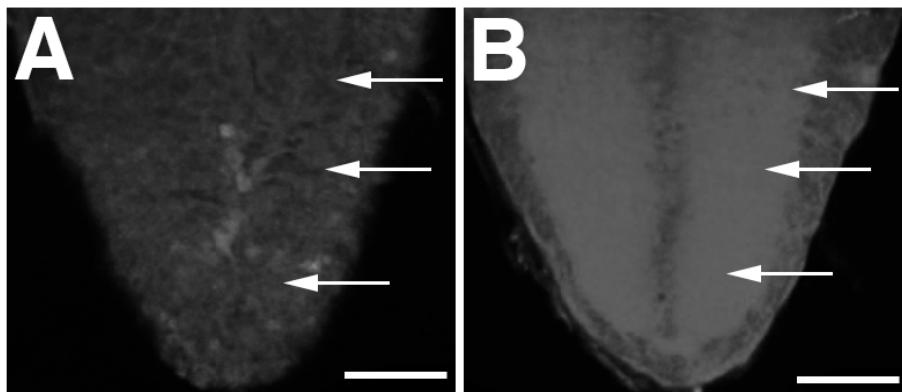
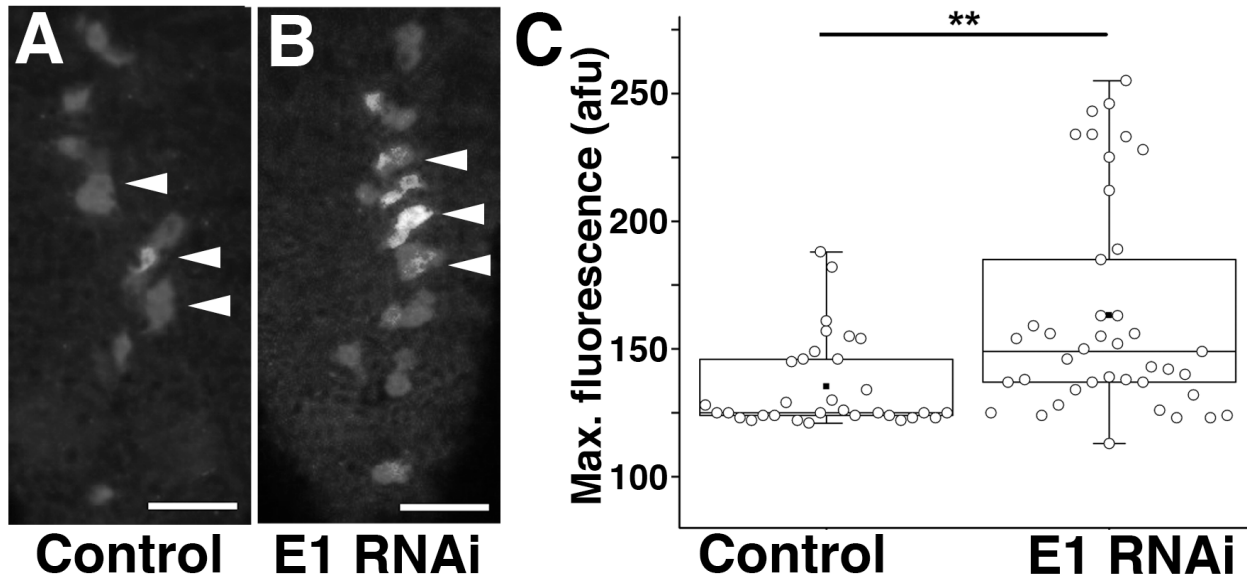


Supplemental Figure 1. Lactacystin inhibits proteasome activity in octopaminergic neurons.

Octopaminergic neurons expressing the GFP-labeled *UAS-degron* and incubated in 100 μ M lactacystin (45 min) show higher levels of fluorescence than cells treated in parallel with vehicle (DMSO) alone. Maximum fluorescence was recorded from individual cells in each nerve cord (Mann-Whitney, $p < 0.05$, 6 animals per treatment, 3 separate experiments, 1 to 14 cells per animal). Box and whiskers represent minimum, first quartile, median (line), mean (solid grey circle), third quartile and maximum.



Supplemental Figure 2. Pan-neuronal E1 RNAi expression decreases proteasome activity in the larval nerve cord. A) Flies pan-neuronally expressing the GFP-tagged *UAS-degron* construct show minimal labeling of the neuropil in the ventral nerve cord (representative image for 6 of 7 animals from 3 experiments; 1 additional sample showed weak labeling of the neuropil). B) Larvae co-expressing *UAS-E1-RNAi* and the *UAS-degron* show widespread fluorescent labeling in the neuropil (representative image for 5 of 6 animals from 3 experiments; 1 other sample showed weaker labeling of the neuropil). Posterior segments are shown in both images, with the terminus of the nerve cord oriented downward in each panel. Arrows in A and B indicate comparable areas. Scale bars: 50 microns.



Supplemental Figure 3. E1 RNAi expression in octopaminergic neurons inhibits proteasome activity. Octopaminergic neurons expressing a GFP labeled *UAS-degron* alone (A) show lower levels of fluorescence than those co-expressing the *UAS-degron* plus a *UAS-E1 RNAi* transgene (B). The midline of the nerve cord terminus is shown in each panel. Arrowheads indicate cell clusters. Scale bars: 25 microns. C) Maximum fluorescence (arbitrary units or afu) was quantified from individual cells in each nerve cord (Mann-Whitney, $p < 0.005$, ≥ 3 animals per treatment, 7 to 18 cells per animal, 3 separate experiments). Box and whiskers represent minimum, first quartile, median (line), mean (solid grey circle), third quartile and maximum.

Supplemental Methods. For all Supplemental Experiments, flies expressing the degron transgene *UAS-GFP CLI* alone, or both *UAS-GFP CLI* and *UAS-E1 RNAi* were crossed to driver lines expressing either *elav-GAL4* (Supplemental Figure 1) or *Tdc2-GAL4* (Supplemental Figures 2 and 3). F_1 progeny were collected as third instar larvae and larval fillets dissected in chilled HL3.1. For Supplemental Figure 1, the fillet was then pre treated in either 100 μ M lactacystin (45 min, 18°C) or vehicle alone (DMSO) prior to fixation. For all experiments, tissue was fixed in 4% paraformaldehyde (25 min) washed 3x in PBS and immersed in 70% glycerol for mounting. Confocal stacks (1024 x 1024 pixels) were collected on a Zeiss Pascal LSM 5 confocal microscope using a 20x/0.8 Plan-Apochromat objective and a pinhole size of 1 Airy unit. Identical laser intensity and gain settings were used for all samples and conditions in each experiment To quantify fluorescence intensity, Regions of Interest (ROIs) were traced around individual cells using Image J, and the maximum level of fluorescence (arbitrary units or “afu”) within each ROI was plotted.